

# Jasmonic acid carboxyl methyltransferase regulates development and herbivory-induced defense response in rice

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**Abstract** Jasmonic acid (JA) and related metabolites play a key role in plant defense and growth. JA carboxyl methyltransferase (JMT) may be involved in plant defense and development by methylating JA to methyl jasmonate (MeJA) and thus influencing the concentrations of JA and related metabolites. However, no JMT gene has been well characterized in monocotyledon defense and development at the molecular level. After we cloned a rice JMT gene, *OsJMT1*, whose encoding protein was localized in the cytosol, we found that the recombinant *OsJMT1* protein catalyzed JA to MeJA. *OsJMT1* is up-regulated in response to infestation with the brown planthopper (BPH; *Nilaparvata lugens*). Plants in which *OsJMT1* had been overexpressed (oe-JMT plants) showed reduced height and yield. These oe-JMT plants also exhibited increased MeJA levels but reduced levels of herbivore-induced JA and jasmonoyl-isoleucine (JA-Ile). The oe-JMT plants were more attractive to BPH female adults but showed increased resistance to BPH nymphs,

probably owing to the different responses of BPH female adults and nymphs to the changes in levels of H<sub>2</sub>O<sub>2</sub> and MeJA in oe-JMT plants. These results indicate that *OsJMT1*, by altering levels of JA and related metabolites, plays a role in regulating plant development and herbivore-induced defense responses in rice.

**Keywords:** Herbivore-induced plant defense; jasmonic acid; jasmonic acid carboxyl methyltransferase; jasmonoyl-isoleucine; methyl jasmonate; *Nilaparvata lugens*; rice

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## INTRODUCTION

Methylation, which is catalyzed by methyltransferases (MTs), is highly conserved in plants and is essential for secondary metabolite modification, protein repair, signal transduction, and developmental and defense processes (Schubert et al. 2003; Qu et al. 2010; Zhao et al. 2010). On the basis of the atom to which the methyl group is attached, MTs are referred to as C-, N-, S-, and O-methyltransferases. The family of SABATH MTs, the name of which is derived from the names of three enzymes, salicylic acid methyltransferase, benzoic acid methyltransferase, and theobromine synthase (Ross et al. 1999; Murfitt et al. 2000; Qu et al. 2010), is a group of O-methyltransferases; these O-methyltransferases are specific to the methylation of carboxyl groups of small molecules and nitrogen atoms of some alkaloids. SABATH MTs are distinct from the other MTs in their sequence characteristics and protein structures (Qu et al. 2010), and are involved in the methylation of several phytohormones, including jasmonic acid (JA), salicylic acid (SA), indole acetic acid, and gibberellins, all of which regulate plant defense responses and developmental and physiological processes (Seo et al. 2001; Pott et al. 2004; Effmert et al. 2005; Qin et al. 2005; Yang

et al. 2006; Koo et al. 2007; Varbanova et al. 2007). Methylation will change the activity, volatility and mobility of these phytohormones, and thus affects their biological functions. Silencing *NtSMT1* in tobacco (*Nicotiana tabacum* L.), for instance, decreases the level of methyl salicylic acid (MeSA) and blocks the plant's systemic acquired resistance (SAR) (Park et al. 2007). Overexpression of *OsBSMT* (benzoic acid (BA) and SA methyltransferase (BSMT)) in *Arabidopsis* reduces disease resistance; such plants have increased MeSA but decreased SA levels after pathogen induction, and MeSA alone cannot induce defense responses in the absence of SA (Koo et al. 2007).

JA, one of the phytohormones that plays a central role in plant defense and growth (Howe and Jander 2008), can be methylated by jasmonic acid carboxyl methyltransferase (JMT), a member of SABATH MTs, to produce methyl jasmonate (MeJA) (Browse and Howe 2008). Like JA, MeJA has been reported to be involved in plant defense, development and seed production (Kobayashi et al. 2010; Darras et al. 2011; Sampedro et al. 2011; Yang et al. 2011). In many plant species, such as *Arabidopsis* and tomato, exogenously applied MeJA induces resistance to spider mites, aphids, and nematodes (Rohwer and Erwin 2010; Fujimoto et al. 2011),

although some studies suggest that this elicitation was mainly due to JA, the hydrolyzed product of MeJA (Wu et al. 2008). The overexpression of *AtJMT* in *Arabidopsis* induces both the plant's constitutive expression of genes related to the biosynthesis of JA and its resistance to *Botrytis cinerea* (Seo et al. 2001). In contrast, *AtJMT* overexpression in *Nicotiana attenuata* does not change the expression levels of genes related to the biosynthesis of JA, but it does reduce induced JA and jasmonoyl-isoleucine (JA-Ile) levels and resistance to herbivores (Seo et al. 2001; Stitz et al. 2011a). Furthermore, overexpressing *AtJMT* in rice plants increases MeJA and abscisic acid (ABA) levels, resulting in drought stress-induced phenotypes and loss of grain yield (Kim et al. 2009). These findings suggest that JMTs influence plant defenses against biotic and abiotic stresses by influencing the products of the oxylipin pathway, such as JA and MeJA, and this effect may be specific to individual plant species. However, to date, studies on JMTs have mainly focused on dicotyledons, and the function of JMTs in monocotyledon defense and growth remains largely unknown.

Rice, one of the most important food crops in the world, suffers heavily from insect pests (Cheng and He 1996). Previous studies have shown that herbivore attack induces a variety of plant hormones in rice, such as JA, SA and ethylene. These subsequently regulate defensive responses, including the release of herbivore-induced plant volatiles (HIPVs) and the accumulation of trypsin proteinase inhibitors (TrypPIs), polyphenol oxidase, and peroxidase (Lou et al. 2005a, 2005b; Lu et al. 2006; Zhou et al. 2009; Lu et al. 2011; Xin et al. 2012; Ye et al. 2012). The exogenous application of MeJA on rice elicits the production of TrypPIs (Lu et al. 2011). However, the biological functions of JMT genes in rice, especially the roles of these genes in plant defense, have not yet been elucidated. In this study, we isolated a rice JMT gene *OsJMT1*, which was induced after infestation with *Spodoptera frugiperda* larvae (Yuan et al. 2008), and obtained rice transgenic lines that overexpress *OsJMT1* (referred to as oe-JMT lines). We determined the role of *OsJMT1* in rice development and grain yield as well as its function in plant defense by measuring herbivore-induced JA, JA-Ile, MeJA and H<sub>2</sub>O<sub>2</sub> levels, and resistance to a phloem feeder (brown planthopper (BPH); *Nilaparvata lugens*). The results indicate that *OsJMT1* may affect rice development and herbivore defense by influencing JA and related metabolites.

## RESULTS

### Isolation and characterization of rice *OsJMT1*

The SABATH family in rice consists of 41 genes distributed on seven chromosomes (Zhao et al. 2008). Using the deduced amino acid sequence of *AtJMT* (AY008434), we searched the GenBank database and identified only one sequence (TIGR ID: Os05g01140, GenBank accession no. AK240953) in rice that showed high similarity with *AtJMT*. Using RT-PCR, we cloned the full-length cDNA of Os05g01140. Phylogenetic analyses were carried out with identified SABATH genes from different plant species. Os05g01140 belongs to the JMT gene group (Figure 1A). Enzymatic analyses using crude protein purified from *Escherichia coli* BL21 carrying the empty vector pET28a showed that when JA was used as the substrate in the

presence of S-adenosyl-L-methionine (SAM), only indole was detected in the reaction products (Figure 1B). When the purified recombinant protein Os05g01140 (for vector see Figure S1A) was used, a large amount of MeJA was detected in the reaction product (Figure 1C). Thus, Os05g01140 has JMT activity and was designated *OsJMT1*.

To clarify the subcellular localization of *OsJMT1*, we constructed an *OsJMT1::EGFP* fusion gene, driven by the CaMV35S promoter (Figure S1B) and transiently expressed the construct in *N. tabacum* leaves. Fluorescence analysis indicated that *OsJMT1* was localized in the cytosol (Figure S2).

Analysis by qRT-PCR revealed that *OsJMT1* transcript levels were rapidly up-regulated by mechanical wounding (Figure 2A). Infestation with BPH gravid female adults induced *OsJMT1* expression strongly (Figure 2B, C), whereas BPH nymph infestation and JA treatment induced *OsJMT1* expression weakly (Figure 2B, C). Treatment with SA slightly decreased *OsJMT1* expression 24 h after treatment (Figure 2D). These results indicated that *OsJMT1* might be involved in herbivore-induced defense responses in rice.

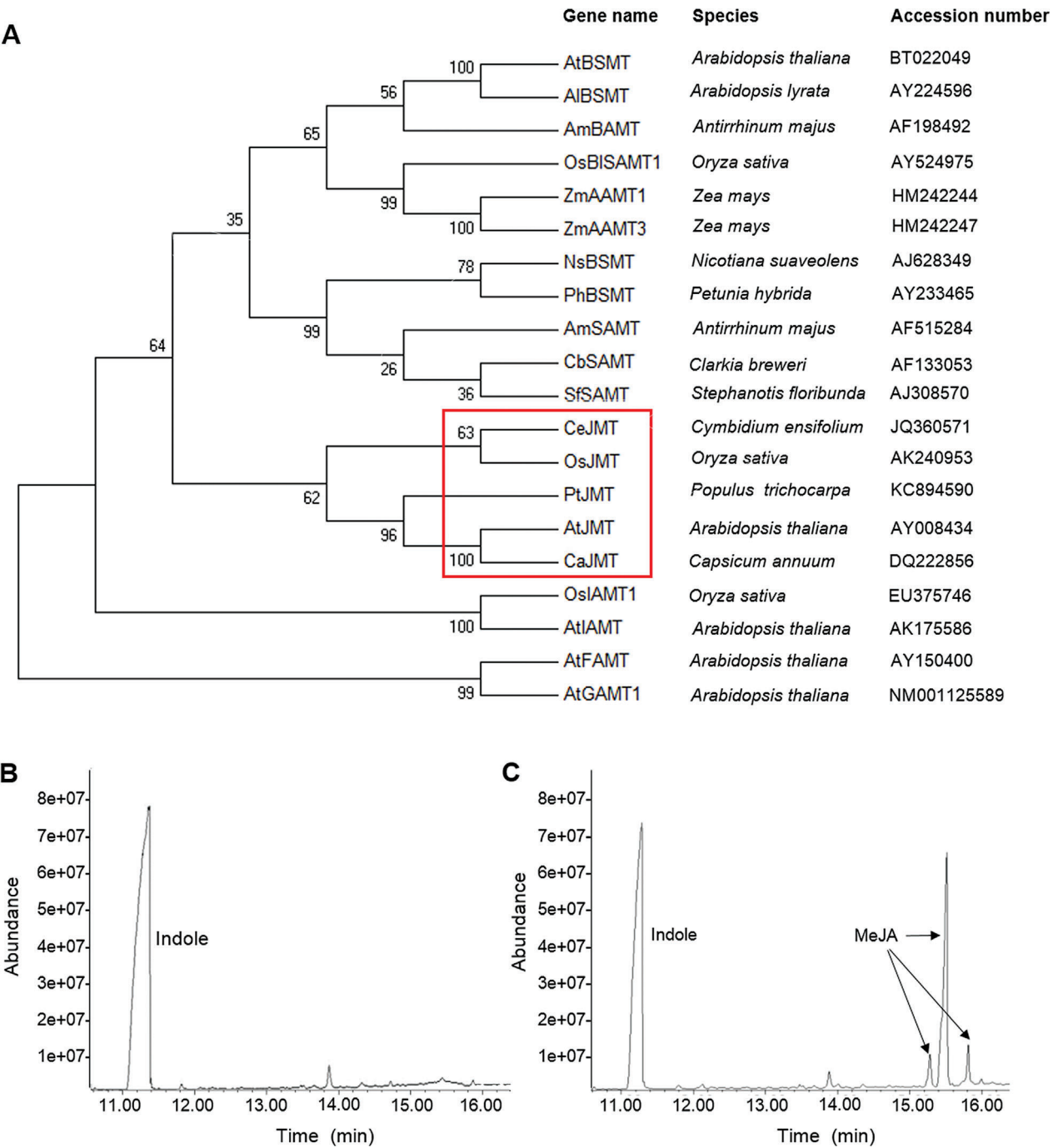
### Overexpression of *OsJMT1* reduces plant height and grain yield

To determine the role of *OsJMT1* in rice development and herbivore-induced defense responses, we constructed the pCambia-1301 transformation vector carrying the full-length open reading frame (ORF) of *OsJMT1* (Figure S1C). Transgenic rice plants were generated using *Agrobacterium tumefaciens*-mediated transformation. By hygromycin resistance and GUS staining selection, we obtained three T<sub>2</sub> homozygous lines (L9-24, L9-64, and L9-70) with a single T-DNA insertion (Figure S3A). Transcriptional analysis showed that transcript levels of *OsJMT1* in L9-24 and L9-70 were significantly higher than those in WT plants (Figure S3B).

Although seedling height was unaffected by the overexpression of *OsJMT1* before the heading stage, oe-JMT plants were shorter at the mature stage compared with WT plants (Figure S4). The overexpression of *OsJMT1* did not affect the number of panicles per plant, but panicle length, number of spikelets per panicle, and the frequency of seed setting were significantly reduced, which led to a reduced total seed weight per plant (Figure 3A–E). Possibly owing to the lower seed setting rate, the weight of 1,000 seeds of L9-24 was slightly higher than that of 1,000 seeds in WT plants (Figure 3F).

### *OsJMT1* mediates herbivore-induced biosynthesis of MeJA, JA, JA-Ile and H<sub>2</sub>O<sub>2</sub>

Phytohormone analysis showed that the basal and gravid BPH female adult-induced MeJA levels were higher in oe-JMT plants than in WT plants (Figure 4A). Basal JA and JA-Ile levels were not significantly different in oe-JMT and WT plants, whereas 3 and 8 h after infestation with gravid BPH female adults, JA and JA-Ile levels in oe-JMT lines were lower than those in WT plants (Figure 4B, C). H<sub>2</sub>O<sub>2</sub> has been reported to be regulated by the JA-signaling pathway and to play an important role in mediating the resistance of rice to BPH (Zhou et al. 2009). Thus, we also investigated the H<sub>2</sub>O<sub>2</sub> levels in oe-JMT lines after BPH infestation. Results showed that the basal H<sub>2</sub>O<sub>2</sub> levels were the same in oe-JMT and in WT plants, but after infestation with gravid BPH female adults, H<sub>2</sub>O<sub>2</sub> levels in oe-JMT plants were significantly lower than in WT plants (Figure 4D).

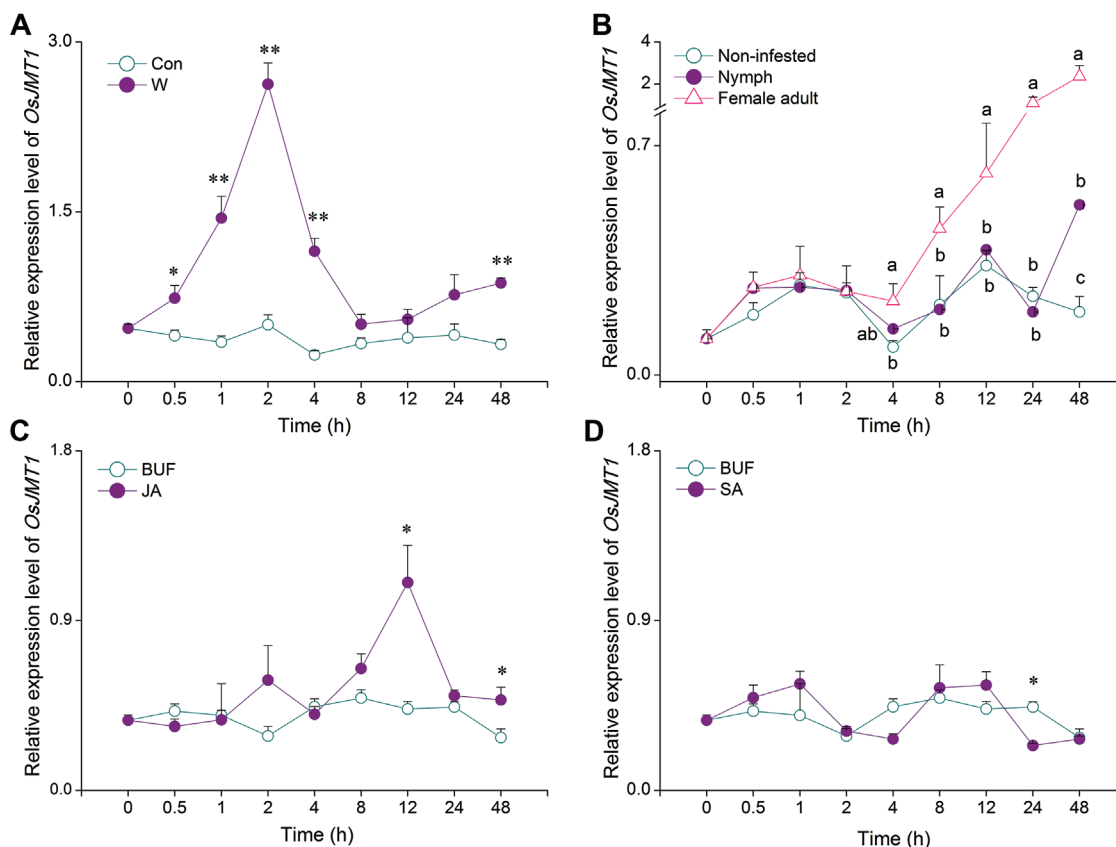


**Figure 1. Phylogenetic relationships of SABATH methyltransferases (MTs) and enzyme activity of OsJMT1**  
(A) Neighbor-joining tree derived from the deduced amino acid sequences of SABATH MTs from different plant species constructed using MEGA5. OsJMT1 and the group of JMTs are enclosed in the red box. (B, C) Reaction products with JA as substrate purified from vector control proteins (B) or OsJMT1 recombinant proteins (C) analyzed by GC-MS.

**OsJMT1 differently mediates resistance to BPH nymphs and female adults**

When different rice genotypes were exposed to a BPH colony, BPH female adults were more frequently observed on oe-JMT lines than on WT plants (Figure 5A, B). Similarly, BPH female adults laid significantly more eggs on oe-JMT lines than on WT plants (Figure 5A and B insert). However, BPH nymphs

preferred to feed on WT plants over oe-JMT lines (Figure 5C, D), and BPH nymphs that fed on WT plants showed a higher survival rate compared to those that fed on oe-JMT lines (Figure 5E). To verify if oe-JMT plants affected other performance parameters in BPH, we also measured the hatching rate and developmental duration of eggs, the developmental duration of nymphs, sex ratio, and the fecundity of female



**Figure 2. Mean transcript levels ( $\pm$ SE;  $n = 5$ ) of *OsJMT1* in rice stems in response to different treatments**

(A) Mechanically wounded (W) and control (Con) plants, (B) infested plants with BPH female adults and with nymphs, and non-infested plants, (C and D) plants treated with jasmonic acid (C) or salicylic acid (D) and sodium phosphate buffer (BUF). Expression levels are relative to those of *OsACT*. Asterisks indicate a significant difference between treatments and controls ( $*P < 0.05$ , Student's *t*-test). Letters within the same time point indicate a significant difference among treatments ( $P < 0.05$ , Duncan's multiple range test).

adults on *oe-JMT* and WT plants. WT plants and *oe-JMT* lines showed no significant difference when these parameters were compared (Figure S5).

To investigate the reason why *oe-JMT* lines affected BPH nymphs and female adults differently, we conducted the following complementation experiments. Applying different amounts of MeJA to WT plants did not change the feeding preference of BPH nymphs (Figure 6A–D), and BPH nymphs showed no preference between WT plants that were treated with MeJA or with lanolin (Figure 6E, F). However, BPH nymphs preferred *oe-JMT* lines treated with lanolin over *oe-JMT* lines treated with MeJA (Figure 6G–J). Unlike BPH nymphs, BPH female adults preferred *oe-JMT* lines treated with lanolin over MeJA-treated WT plants (Figure 7A, B). WT plants and *oe-JMT* lines treated with MeJA attracted more BPH female adults than did WT plants and *oe-JMT* lines treated with lanolin only (Figure 7C–E).

## DISCUSSION

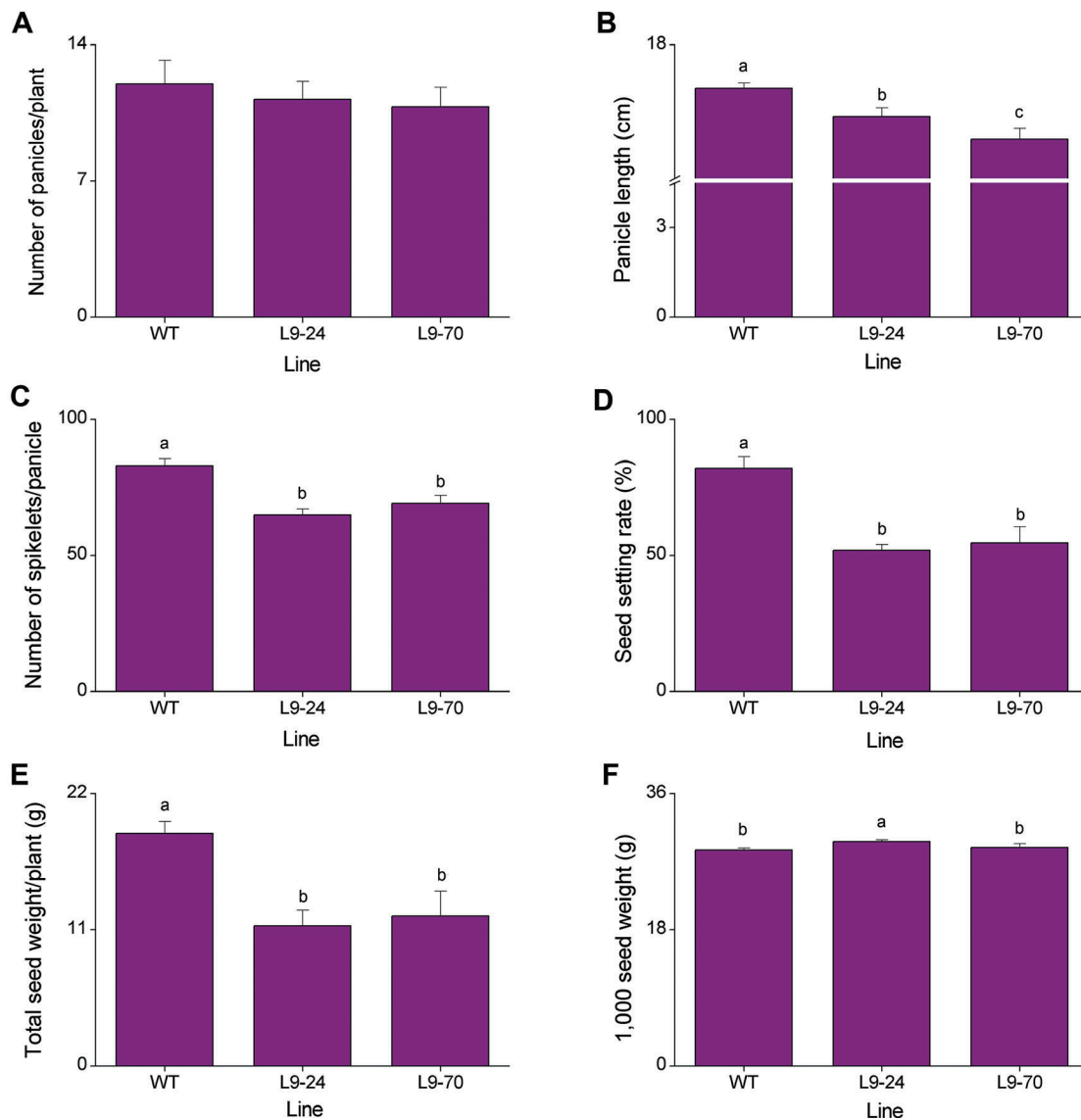
We cloned a rice gene *OsJMT1* that possesses JMT activity. The overexpression of *OsJMT1* increased basal and BPH-induced

levels of MeJA, but decreased herbivore-induced levels of JA and JA-Ile in rice, which in turn reduced plant growth and yield. Moreover, *oe-JMT* lines were susceptible to BPH female adults but were resistant to BPH nymphs. These findings support the notion that the alteration in JA and related metabolites plays an important role in plant growth and defense.

### *OsJMT1* and its regulation of defense-related signals

Transcriptional analysis revealed that BPH female adults and nymphs had different effects on how *OsJMT1* was induced: BPH female adults induced *OsJMT1* transcription faster and more strongly than BPH nymphs (Figure 2). This difference demonstrates that specific signals from BPH eggs may elicit the expression of *OsJMT1*. Signals from herbivore eggs have been well documented to induce plant defense (Hilker and Fatouros 2015). Such signals have been identified in the eggs of the rice white-backed planthopper *Sogatella furcifera*, for example; these signals are lipids and can induce rice plants to produce benzyl benzoate, which in turn kills *S. furcifera* eggs (Yang et al. 2013). The signals from BPH eggs should be exploited in the future.

We found that *oe-JMT* plants had higher basal and herbivore-induced MeJA levels and lower JA and JA-Ile levels



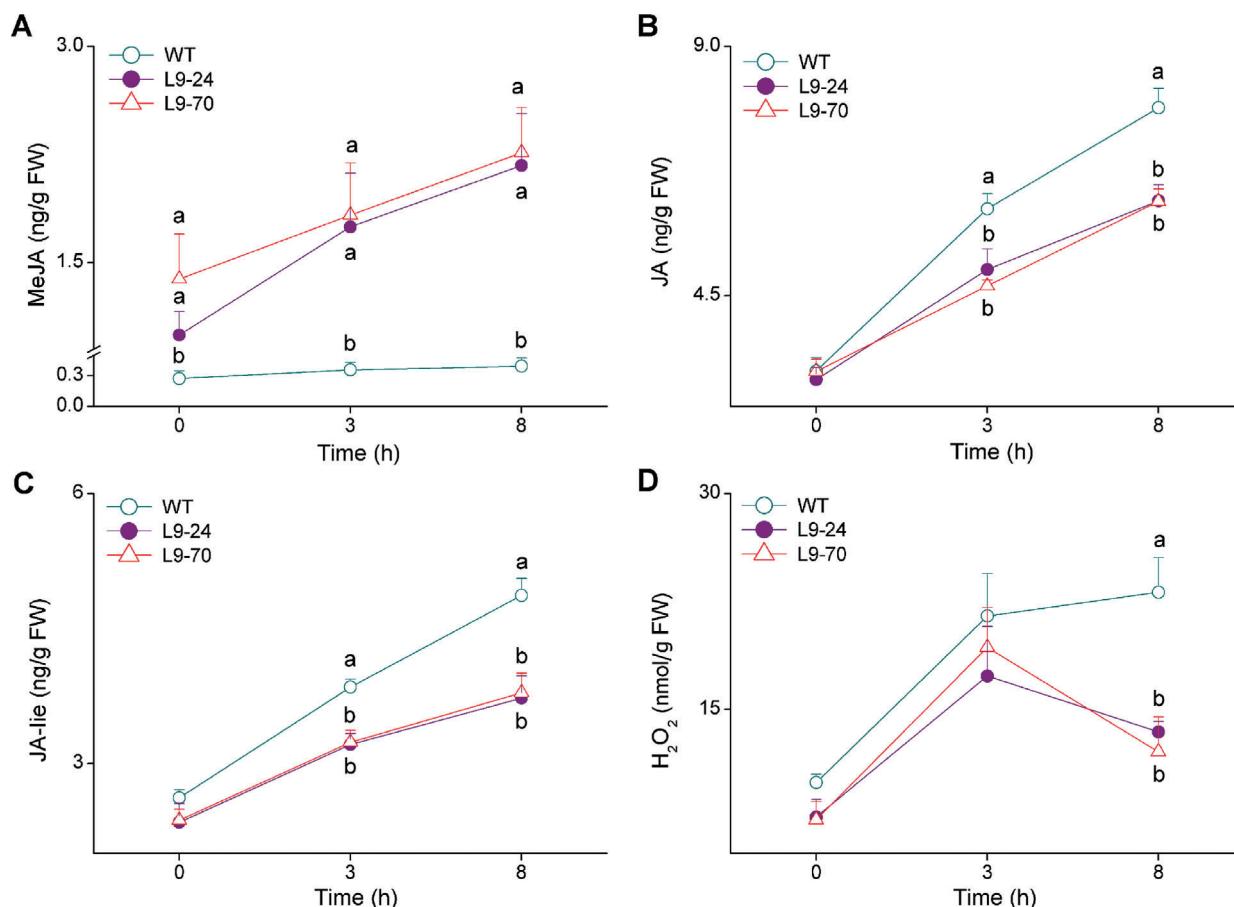
**Figure 3. Rice yield production parameters in wild-type (WT) plants and oe-JMT lines**

The values present mean  $\pm$  SE ( $n = 10$ ). Letters indicate significant differences among lines ( $P < 0.05$ , Duncan's multiple range test).

compared to WT plants treated the same way (Figure 4A–C). Similarly, when attacked by the rice striped stem borer (SSB) *Chilo suppressalis*, oe-JMT plants showed higher MeJA but lower JA levels compared to WT plants; moreover, higher elicited transcript levels of *OsAOS1* and *OsHI-LOX* were observed in oe-JMT plants compared to in WT plants (Figure S6). These results were not fully consistent with the results found in other plant species. Arabidopsis and rice plants that overexpress *AtJMT*, for example, did not show reduced JA levels (Seo et al. 2001; Kim et al. 2009). Similarly, the overexpression of *AtJMT* in *N. attenuata* had no influence on the induced transcript levels of genes related to JA biosynthesis (Stitz et al. 2011b). This suggests that the manipulation of JA and related metabolites by JMT may have different results depending on plant species and specific JMTs. The findings suggest that *OsJMT1*, in addition to

methylation JA to produce MeJA and thus altering the levels of JA and related metabolites, may also change the levels of JA-related metabolites by regulating the activity of enzymes in the JA biosynthesis pathway. Future research should elucidate whether or which other signals in the JA pathway, such as 12-oxo-phytodienoic acid (OPDA), were affected in the oe-JMT plants.

In addition to inducing plants with JA, JA-Ile and MeJA, we also observed that the BPH-induced  $H_2O_2$  level in oe-JMT plants was significantly reduced compared to that in WT plants (Figure 4D). Lower JA levels were observed in oe-JMT lines than in WT plants, which was unlike our previous result, namely, that the antisense expression of *OsHI-LOX* (*as-lox*) in rice decreased herbivore-elicited JA levels but enhanced  $H_2O_2$  levels (Zhou et al. 2009). However, unlike *as-lox* lines in which *OsHI-LOX* was silenced, oe-JMT lines



**Figure 4.** Mean levels ( $\pm$ SE;  $n = 5$ ) of methyl jasmonate (MeJA), jasmonic acid (JA), jasmonoyl-isoleucine (JA-Ile), and  $H_2O_2$  in stems of two oe-JMT lines and WT plants 0, 3 and 8 h after BPH infestation

Letters indicate significant differences among lines ( $P < 0.05$ , Duncan's multiple range test). FW, fresh weight.

increased transcript levels of *OsHI-LOX* (Figure S6). This discrepancy demonstrates that the elicited  $H_2O_2$  levels in rice might be related to the activity of *OsHI-LOX* and/or its mediated signals rather than to the presence of JA. A similar result has been reported in cotton, where a 9-lipoxygenase *GhLOX1* gene was found to be associated with the hypersensitive reaction of cotton to *Xanthomonas campestris* pv *malvacearum* (Marmey et al. 2007).

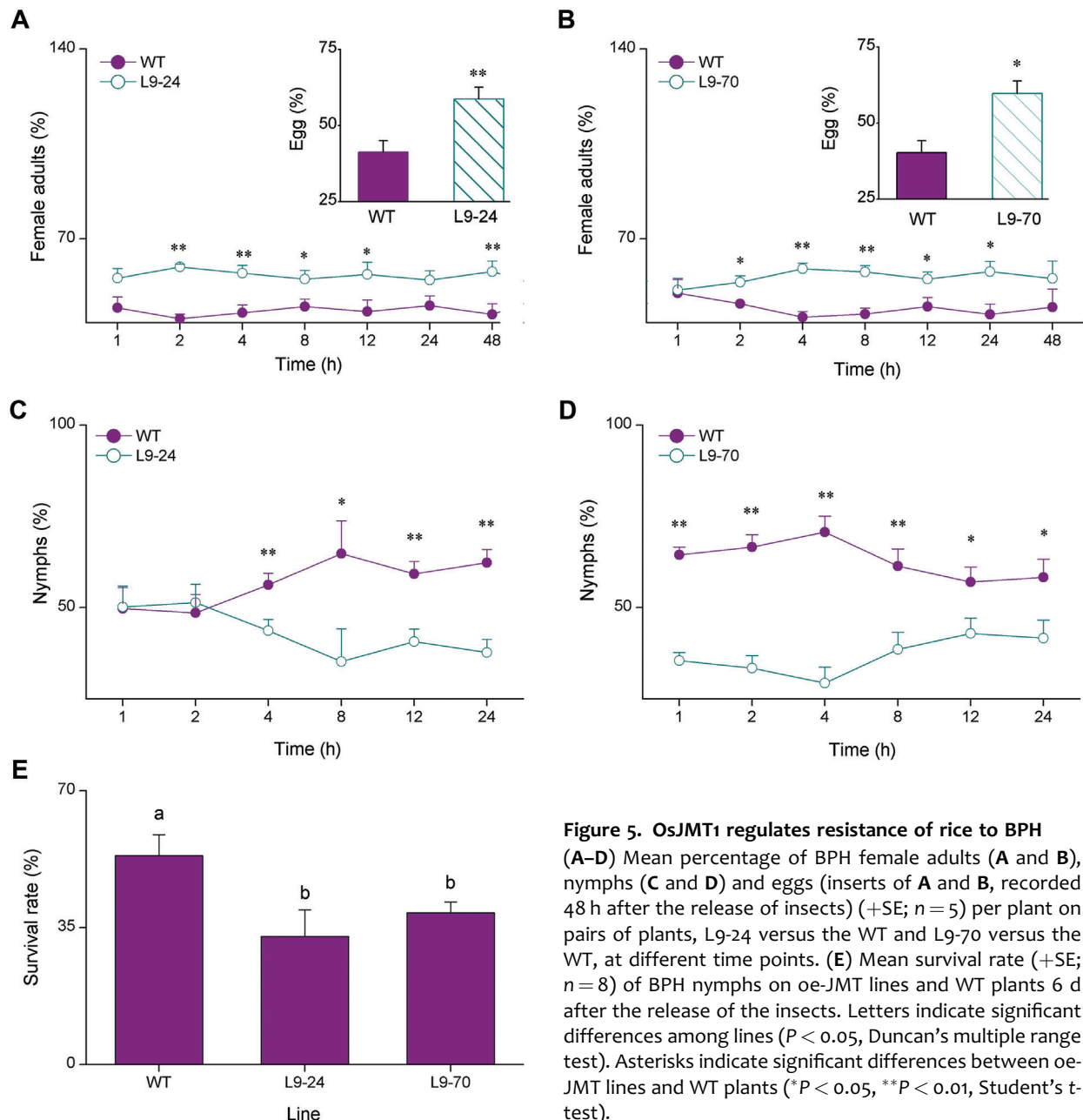
#### Effects of *OsJMT1* on rice growth and yield

Both JA and MeJA have been reported to regulate plant growth and seed production. Mutant *Arabidopsis* plants, for instance, that had decreased levels of JA or that were insensitive to JA (such as *fad3-1/fad7-2/fad8* and *coi1*) had impaired floral development; in addition, the ability of mutant plants to release pollen was negatively affected (McConn 1996; Xie et al. 1998; Sanders et al. 2000; Ishiguro et al. 2001). Similarly, the overexpression of *AtJMT* in *Arabidopsis* resulted in high MeJA levels but unchanged JA levels, as well as in decreased seed mass and seed number (Cipollini 2010). Moreover, rice with overexpressed *AtJMT*, which increased MeJA levels but left its JA levels unchanged, induced high ABA levels and thus resulted in reduced grain yield and seedling height (Kim et al. 2009). Therefore, as was found in rice

overexpressing *AtJMT* (Kim et al. 2009), the reduced growth and yield observed in oe-JMT lines may be related to these lines' low JA and/or high MeJA levels, especially the latter. Using different mutants with impaired or increased JA or MeJA levels may help researchers dissect the role of signals in both plant growth and reproduction. JMT has been shown to be expressed in reproductive organs and its expression is developmentally regulated (Seo et al. 2001). Thus, to explore the mechanism underlying the *OsJMT1* regulation of rice growth and reproduction, it will also be important to investigate the expression of *OsJMT1* in different tissues and to determine whether the expression of *OsJMT1* influences levels of JA and related metabolites in reproductive organs.

#### Possible mechanisms of *OsJMT1* as a regulator of plant defense against herbivores

In the BPH bioassay, we observed that BPH nymphs preferred WT plants to oe-JMT plants, whereas BPH female adults preferred oe-JMT lines to WT plants (Figure 5A–D). Moreover, BPH nymphs had a lower survival rate on oe-JMT plants than on WT plants (Figure 5E). These effects could result from: (i) the MeJA-elicited defense responses; (ii) the increased MeJA itself; (iii) the reduced  $H_2O_2$ ; or (iv) the defense responses

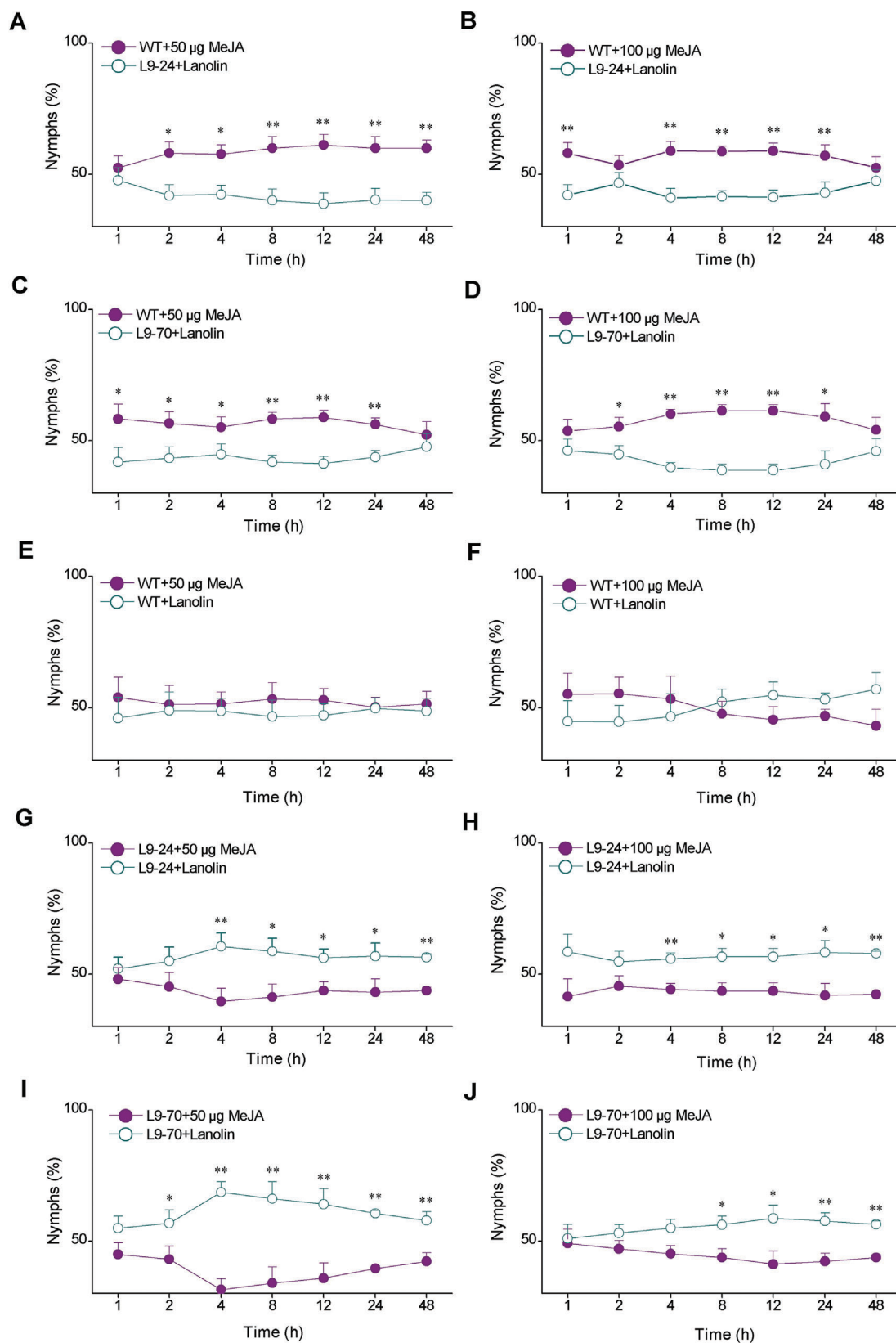


**Figure 5. OsJMT1 regulates resistance of rice to BPH**

(A–D) Mean percentage of BPH female adults (A and B), nymphs (C and D) and eggs (inserts of A and B, recorded 48 h after the release of insects) (+SE;  $n = 5$ ) per plant on pairs of plants, L9-24 versus the WT and L9-70 versus the WT, at different time points. (E) Mean survival rate (+SE;  $n = 8$ ) of BPH nymphs on oe-JMT lines and WT plants 6 d after the release of the insects. Letters indicate significant differences among lines ( $P < 0.05$ , Duncan's multiple range test). Asterisks indicate significant differences between oe-JMT lines and WT plants (\* $P < 0.05$ , \*\* $P < 0.01$ , Student's  $t$ -test).

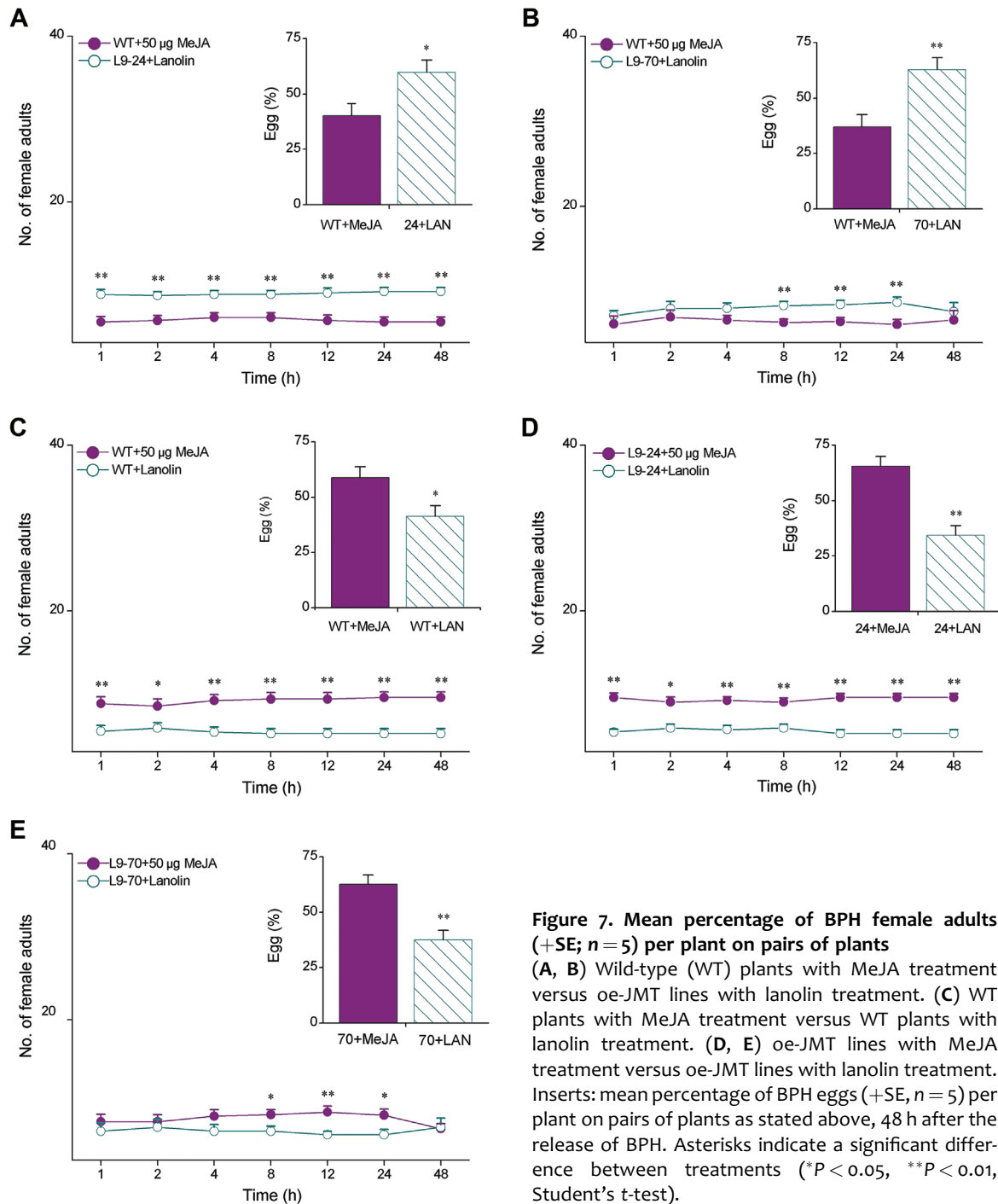
induced by signals in the JA pathway (JA-Ile, for example) other than those elicited by MeJA. Recently, in *N. attenuata*, it has been found that MeJA itself is not a defense-related signal and that MeJA-induced resistance is elicited by JA (Wu et al. 2008). Thus, the first hypothesis can be ruled out. BPH nymphs were not sensitive to MeJA-treated WT plants (Figure 6E, F) but were repelled by oe-JMT plants treated with MeJA (Figure 6G–J). BPH female adults preferred oe-JMT plants to WT plants even when WT plants were treated with MeJA, whereas these insects preferred MeJA-treated WT plants or treated oe-JMT lines over their corresponding control plants (Figure 7C–E). Given that MeJA treatment increased MeJA levels in oe-JMT plants but not in WT plants (Figure S7) and that MeJA may not be a defense-related signal, we suggest that MeJA itself, a volatile chemical at ambient temperatures,

is attractive to BPH female adults but repellent and/or toxic to BPH nymphs. The effect of MeJA on herbivores has also been reported in other plant-herbivore systems. Under wild conditions, for example, sagebrush produces high amounts of MeJA and is not attacked by mirids (Dinh et al. 2013). MeJA treatment may have increased MeJA levels only in oe-JMT plants, because the levels of JA and MeJA in WT plants are kept in balance by JMT and MJE (exogenous MeJA is hydrolyzed to JA by MJE). However, in oe-JMT plants, the balance is disrupted by the overexpression of *OsJMT1*; such overexpression methylates JA to produce and maintain high MeJA levels (Howe and Jander 2008). Hydrogen peroxide has a negative effect on survival rates, and on the feeding and ovipositing of BPHs (Zhou et al. 2009; Lu et al. 2011). Therefore, the lower elicited  $H_2O_2$  level in oe-JMT plants may



**Figure 6. Mean percentage of BPH nymphs (+SE;  $n = 5$ ) per plant on pairs of plants**

(A–D) Wild-type (WT) plants with MeJA treatment versus oe-JMT lines with lanolin treatment. (E, F) WT plants with MeJA treatment versus WT plants with lanolin treatment. (G–J) oe-JMT lines with MeJA treatment versus oe-JMT lines with lanolin treatment. Asterisks indicate significant differences between treatments (\* $P < 0.05$ , \*\* $P < 0.01$ , Student's  $t$ -test).



**Figure 7. Mean percentage of BPH female adults (+SE;  $n = 5$ ) per plant on pairs of plants (A, B) Wild-type (WT) plants with MeJA treatment versus oe-JMT lines with lanolin treatment. (C) WT plants with MeJA treatment versus WT plants with lanolin treatment. (D, E) oe-JMT lines with MeJA treatment versus oe-JMT lines with lanolin treatment. Inserts: mean percentage of BPH eggs (+SE,  $n = 5$ ) per plant on pairs of plants as stated above, 48 h after the release of BPH. Asterisks indicate a significant difference between treatments (\* $P < 0.05$ , \*\* $P < 0.01$ , Student's t-test).**

also be a reason why BPH female adults preferred oe-JMT plants over WT plants. Further research should elucidate these possibilities.

In summary, *OsJMT1*, by mediating the levels of JA and related metabolites, plays an important role in plant growth and defense in rice. In JA and related metabolites, MeJA may be a signal that regulates plant growth and development but not defense, whereas JA and other signals, such as JA-Ile, may mainly mediate defense. BPH nymphs and female adults may be affected by  $H_2O_2$  and MeJA itself. Given that *OsJMT1* was induced strongly by BPH female adults but

weakly by nymphs and that MeJA is beneficial to female adults but harmful to nymphs, BPH seems to have the capacity to cope with defense responses in rice by regulating *OsJMT1*.

## MATERIALS AND METHODS

### Plant growth

The rice (*Oryza sativa*) genotypes used in this study were cultivar Xiushui 110 as the wild-type (WT) and oe-JMT

transgenic lines (see below). Pre-germinated seeds were cultured in plastic bottles (diameter, 8 cm; height, 10 cm) in a greenhouse ( $28 \pm 2^\circ\text{C}$ , 14/10 h (light/dark) photoperiod). Ten-day-old seedlings were transferred to 20 L communal hydroponic boxes containing a rice nutrient solution (Yoshida et al. 1976). After 40 d, seedlings were transferred to individual 500-mL hydroponic plastic pots, with one or two plants per pot (when two plants, one was a WT plant and the other was a plant from an oe-JMT transgenic line). Plants were used for experiments 4–5 d after transplanting.

### Insects

Colonies of BPH and SSB were maintained on seedlings of rice cultivar Shanyou 63 (a variety susceptible to BPH and SSB) in a controlled climate chamber at  $26 \pm 2^\circ\text{C}$ , with a 12/12 h (light/dark) photoperiod and 80% relative humidity.

### Generation and characterization of oe-JMT lines

The full-length cDNA of *OsJMT1* was obtained by reverse transcription (RT)-PCR from RNA isolated from WT plants that had been attacked by a striped stem borer larva for 24 h. The primers *OsJMT1-F* (5'-GCGTATGATAGATGTCAGAG-3') and *OsJMT1-R* (5'-GAAGGAAGGAAGGATGGGAT-3') were designed on the basis of the sequence of GenBank accession no. AK240953 (TIGR ID Os05g01140). The cDNA was cloned into pCAMBIA1301 to yield an overexpression transformation vector (Figure S1A). The T-DNA was inserted into *Xiushui 110* using *A. tumefaciens*-mediated transformation. The procedures for rice transformation, screening of the homozygous  $T_2$  plants, and identification of the number of insertions followed Qi et al. (2011). For most experiments, WT plants and two  $T_2$  homozygous lines, L9-24 and L9-70, were used.

### Subcellular localization

The full-length ORF without a stop codon for *OsJMT1* cDNA was cloned into the pEGFP vector to fuse with EGFP. The fusion gene, *OsJMT1:EGFP*, was inserted into pCAMBIA1301 to yield a transformation vector (Figure S1B). This vector was used to transiently transform *N. tabacum* leaves. Transformation and fluorescence analysis were performed as described by Lu et al. (2011).

### OsJMT1 activity assay

*OsJMT1* cDNA was inserted into the pET-28a vector at the *Sac* I and *Hind* III sites. The resulting vector (Figure S1C) was transformed into *E. coli* strain BL21. The cell strains containing either the pET-28a vector or the vector carrying *OsJMT1* were cultured in LB media at  $37^\circ\text{C}$  until  $\text{OD}_{600}$  reached 0.6. Then isopropyl  $\beta$ -D-1-thiogalactopyranoside (IPTG) and JA were added to the medium to make a final concentration of 1.0 mM and  $5 \mu\text{g/mL}$ , respectively, and cultured at  $20^\circ\text{C}$  for an additional 20 h. Cells were removed by centrifugation and the supernatant was extracted with 5 mL *n*-hexane. The extract was concentrated to 200  $\mu\text{L}$  and used for enzyme activity analysis by gas chromatography-mass spectrometry (GC-MS).

### Plant treatments

For BPH treatment, plants were individually infested with 25 BPH nymphs or 15 gravid BPH female adults that were confined within a glass cylinder (diameter, 4 cm; height, 8 cm;

with 48 small holes (diameter, 0.8 mm)). One empty cylinder was used to enclose non-infested plants. For SSB treatment, plants were individually infested using a third-instar larva of SSB that had been starved for 2 h. Control plants were free of herbivores. Mechanically wounded plants were individually damaged by a needle on the lower part of each stem (about 2 cm long), and 200 holes were generated. Control plants were not pierced. For JA and SA treatment, plants were individually sprayed with 2 mL JA ( $100 \mu\text{g/mL}$ ) or 2 mL SA ( $70 \mu\text{g/mL}$ ) in 50 mM sodium phosphate buffer. Control plants were sprayed with 2 mL buffer. For MeJA treatment, plants from each genotype were individually treated with 50–100  $\mu\text{g}$  MeJA in lanolin paste (see details for each experiment) on the stem. Control plants received the same volume of pure lanolin paste.

### Quantitative real-time PCR

For quantitative real-time PCR (qRT-PCR) analysis, five independent biological samples were used. Total RNA was isolated using the SV Total RNA Isolation System (Promega). One microgram of each RNA sample was reverse-transcribed using the PrimeScript RT-PCR Kit (Takara). The qRT-PCR analysis was performed on a Biorad CFX96 machine. The *OsACT* gene was used for normalization. The primers and TaqMan probe sequences used for TaqMan qRT-PCR (Premix Ex Taq Kit; Takara) are listed in Table S1.

### JA, JA-Ile and MeJA analysis

Plants (one plant per pot) were randomly assigned to MeJA, BPH infestation, SSB infestation and their corresponding control treatments. Two oe-JMT lines (L9-24 and L90) and WT plants were used. The stems were harvested at 0, 3, and 8 h after BPH infestation, 3 h after SSB infestation and 1 h after MeJA treatment. Samples were ground in liquid nitrogen, and JA and JA-Ile were extracted with ethyl acetate spiked with labeled internal standards ( $^{13}\text{C}_2$ -JA and  $^{13}\text{C}_6$ -JA-Ile, each with 100 ng) following the method as described in Stitz et al. (2011b); MeJA was extracted by 70% methanol spiked with 100 ng of  $^{13}\text{C}_2$ -MeJA (synthesized by the esterification of  $^{13}\text{C}_2$ -JA). After centrifugation at maximum speed for 10 min at  $4^\circ\text{C}$ , the supernatants were collected. JA, JA-Ile and MeJA levels were analyzed by Shimadzu LC/MS-8040 as described in Stitz et al. (2011b).

### Quantification of $\text{H}_2\text{O}_2$

Plants of the WT and oe-JMT lines L9-24 and L9-70 were randomly assigned to the BPH-infested group or the non-infested control group. Leaf sheaths were harvested at 0, 3 and 8 h after the start of treatment. Five replicates per treatment were sampled at each time point. The concentration of  $\text{H}_2\text{O}_2$  was determined as described by Lou and Baldwin (2006).

### Herbivory experiments

To determine the colonization and oviposition preference of BPH, pots containing two plants (an oe-JMT line plant and a WT plant) were confined with glass cylinders into which 15 gravid BPH female adults or nymphs were introduced. In complementation experiments, the feeding and/or oviposition preferences of BPH female adults and nymphs were determined for 15 pairs of plants (each pair contained a MeJA-

treated plant and a lanolin-treated plant; see details in Figure 7) onto which 15 gravid BPH female adults or nymphs were introduced. The numbers of BPH at different times (see details in Figure 5–7) and BPH eggs at 48 h on each plant were counted following the method described by Zhou et al. (2009). The experiment was repeated six times.

To measure the survival rate of BPH nymphs, 15 newly hatched nymphs were individually introduced onto plants of WT and oe-JMT lines, and then we recorded the number of nymphs that were alive 6 d later. To measure the hatching rate of BPH eggs, we released five gravid BPH female adults per plant onto plants that had been confined in a glass cylinder for 1 d. Every day, the number of newly hatched nymphs was recorded, and when no nymphs were seen, the dead eggs were counted under a microscope. Both experiments were repeated 8–10 times. Simultaneously, the newly hatched nymphs were introduced onto new plants; each plant had one nymph, each genotype had 50 nymphs. Every day, the number of emerging adults was recorded until all of the nymphs had become adults. After emergence, pairs of female and male adults were transferred to new plants (one pair per plant), and the number of BPH eggs on each plant was counted after 10 d, following the method described by Zhou et al. (2009). Based on these data, the developmental duration of female and male BPH nymphs ( $n = 40$ – $50$ ) as well as the number of eggs laid per female ( $n = 15$ – $20$ ) were calculated.

#### Data analysis

Differences in all of the data were determined by one-way ANOVA (Student's *t*-test was used for comparison of two treatments). If the ANOVA was significant ( $P < 0.05$ ), Duncan's multiple range test was used to detect significant differences between groups. All tests were carried out with Statistica (SAS Institute Inc., <http://www.sas.com/>).

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## AUTHOR CONTRIBUTIONS

J.Q. and J.L. performed the bioassay and phytohormone analysis and J.Q. drafted the manuscript. X.H. and R.L. generated the Oe-JMT lines and performed OsJMT1 activity assay, and H.Y., L.H. and Y.X. carried out qRT-PCR analysis and subcellular localization. J.W. revised the manuscript. Y.L. designed the experiment, supervised the study, and revised the manuscript.

## REFERENCES

- Browse J, Howe GA (2008) New weapons and a rapid response against insect attack. *Plant Physiol* 146: 832–838
- Cheng J, He J (1996) *Rice Insect Pests*. China Agricultural Press, Beijing
- Cipollini D (2010) Constitutive expression of methyl jasmonate-inducible responses delays reproduction and constrains fitness responses to nutrients in *Arabidopsis thaliana*. *Evol Ecol* 24: 59–68
- Darras AI, Joyce DC, Terry LA (2011) Methyl jasmonate and acibenzolar-S-methyl protect cut *Freesia hybrida* inflorescences against *Botrytis cinerea*, but do not act synergistically. *J Hortic Sci Biotech* 86: 74–78
- Dinh S, Gális I, Baldwin I (2013) UVB radiation and HGL-DTGs provide durable resistance against mirid (*Tupiocoris notatus*) attack in field-grown *Nicotiana attenuata* plants. *Plant Cell Environ* 36: 590–606
- Effmert U, Saschenbrecker S, Ross J, Negre F, Fraser CM, Noel JP, Dudareva N, Piechulla B (2005) Floral benzenoid carboxyl methyltransferases: From *in vitro* to *in planta* function. *Phytochemistry* 66: 1211–1230
- Fujimoto T, Tomitaka Y, Abe H, Tsuda S, Futai K, Mizukubo T (2011) Expression profile of jasmonic acid-induced genes and the induced resistance against the root-knot nematode (*Meloidogyne incognita*) in tomato plants (*Solanum lycopersicum*) after foliar treatment with methyl jasmonate. *J Plant Physiol* 168: 1084–1097
- Hilker M, Fatouros NE (2015) Plant responses to insect egg deposition. *Annu Rev Entomol* 60: 493–515
- Howe GA, Jander G (2008) Plant immunity to insect herbivores. *Annu Rev Plant Biol* 59: 41–66
- Ishiguro S, Kawai-Oda A, Ueda J, Nishida I, Okada K (2001) The DEFECTIVE IN ANOTHER DEHISCENCE1 gene encodes a novel phospholipase A1 catalyzing the initial step of jasmonic acid biosynthesis, which synchronizes pollen maturation, anther dehiscence, and flower opening in *Arabidopsis*. *Plant Cell* 13: 2191–2209
- Kim EH, Kim YS, Park SH, Koo YJ, Do Choi Y, Chung YY, Lee IJ, Kim JK (2009) Methyl jasmonate reduces grain yield by mediating stress signals to alter spikelet development in rice. *Plant Physiol* 149: 1751–1760
- Kobayashi H, Yanaka M, Ikeda TM (2010) Exogenous Methyl jasmonate alters trichome density on leaf surfaces of rhodes grass (*Chloris gayana* Kunth). *J Plant Growth Regul* 29: 506–511
- Koo YJ, Kim MA, Kim EH, Song JT, Jung C, Moon JK, Kim JH, Seo HS, Song SI, Kim JK, Lee JS, Cheong JJ, Do Choi Y (2007) Overexpression of salicylic acid carboxyl methyltransferase reduces salicylic acid-mediated pathogen resistance in *Arabidopsis thaliana*. *Plant Mol Biol Rep* 64: 1–15
- Lou YG, Ma B, Cheng JA (2005a) Attraction of the parasitoid *Anagrus nilaparvatae* to rice volatiles induced by the rice brown planthopper *Nilaparvata lugens*. *J Chem Ecol* 31: 2357–2372
- Lou YG, Du MH, Turlings TCJ, Cheng JA, Shan WF (2005b) Exogenous application of jasmonic acid induces volatile emissions in rice and enhances parasitism of *Nilaparvata lugens* eggs by the parasitoid *Anagrus nilaparvatae*. *J Chem Ecol* 31: 1985–2002
- Lu J, Ju HP, Zhou GX, Zhu CS, Erb M, Wang XP, Wang P, Lou YG (2011) An EAR-motif-containing ERF transcription factor affects herbivore-induced signaling, defense and resistance in rice. *Plant J* 68: 583–596
- Lu YJ, Wang X, Lou YG, Cheng JA (2006) Role of ethylene signaling in the production of rice volatiles induced by the rice brown planthopper *Nilaparvata lugens*. *Chin Sci Bull* 51: 2457–2465
- Marmey P, Jalloul A, Alhamdia M, Assigbetse K, Cacas JL, Voloudakis AE, Champion A, Clerivet A, Montillet JL, Nicole M (2007) The 9-lipoxygenase GhLOX1 gene is associated with the

- hypersensitive reaction of cotton *Gossypium hirsutum* to *Xanthomonas campestris* pv *malvacearum*. **Plant Physiol Biochem** 45: 596–606
- McConn M (1996) The critical requirement for linolenic acid is pollen development, not photosynthesis, in an *Arabidopsis* mutant. **Plant Cell** 8: 403–416
- Murfitt LM, Kolosova N, Mann CJ, Dudareva N (2000) Purification and characterization of S-adenosyl-L-methionine: Benzoic acid carboxyl methyltransferase, the enzyme responsible for biosynthesis of the volatile ester methyl benzoate in flowers of *Antirrhinum majus*. **Arch Biochem Biophys** 382: 145–151
- Park SW, Kaimoyo E, Kumar D, Mosher S, Klessig DF (2007) Methyl salicylate is a critical mobile signal for plant systemic acquired resistance. **Science** 318: 113–116
- Pott MB, Hippauf F, Saschenbrecker S, Chen F, Ross J, Kiefer I, Slusarenko A, Noel JP, Pichersky E, Effmert U, Piechulla B (2004) Biochemical and structural characterization of benzenoid carboxyl methyltransferases involved in floral scent production in *Stephanotis floribunda* and *Nicotiana suaveolens*. **Plant Physiol** 135: 1946–1955
- Qi JF, Zhou GX, Yang LJ, Erb M, Lu YH, Sun XL, Cheng JA, Lou YG (2011) The chloroplast-localized phospholipases d alpha 4 and alpha 5 regulate herbivore-induced direct and indirect defenses in rice. **Plant Physiol** 157: 1987–1999
- Qin GJ, Gu HY, Zhao YD, Ma ZQ, Shi GL, Yang Y, Pichersky E, Chen HD, Liu MH, Chen ZL, Qu LJ (2005) An indole-3-acetic acid carboxyl methyltransferase regulates *Arabidopsis* leaf development. **Plant Cell** 17: 2693–2704
- Qu LJ, Li SA, Xing SF (2010) Methylation of phytohormones by the SABATH methyltransferases. **Chin Sci Bull** 55: 2211–2218
- Rohwer CL, Erwin JE (2010) Spider mites (*Tetranychus urticae*) perform poorly on and disperse from plants exposed to methyl jasmonate. **Entomol Exp Appl** 137: 143–152
- Ross JR, Nam KH, D'Auria JC, Pichersky E (1999) S-adenosyl-L-methionine: Salicylic acid carboxyl methyltransferase, an enzyme involved in floral scent production and plant defense, represents a new class of plant methyltransferases. **Arch Biochem Biophys** 367: 9–16
- Sampedro L, Moreira X, Zas R (2011) Resistance and response of *Pinus pinaster* seedlings to *Hylobius abietis* after induction with methyl jasmonate. **Plant Ecol** 212: 397–401
- Sanders PM, Lee PY, Biesgen C, Boone JD, Beals TP, Weiler EW, Goldberg RB (2000) The *Arabidopsis* DELAYED DEHISCENCE1 gene encodes an enzyme in the jasmonic acid synthesis pathway. **Plant Cell** 12: 1041–1061
- Schubert HL, Blumenthal RM, Cheng XD (2003) Many paths to methyltransfer: A chronicle of convergence. **Trends Biochem Sci** 28: 329–335
- Seo HS, Song JT, Cheong JJ, Lee YH, Lee YW, Hwang I, Lee JS, Choi YD (2001) Jasmonic acid carboxyl methyltransferase: A key enzyme for jasmonate-regulated plant responses. **Proc Natl Acad Sci USA** 98: 4788–4793
- Stitz M, Baldwin IT, Gaquerel E (2011a) Diverting the flux of the ja pathway in *Nicotiana attenuata* compromises the plant's defense metabolism and fitness in nature and glasshouse. **PLoS ONE** 6: e25925
- Stitz M, Gase K, Baldwin IT, Gaquerel E (2011b) Ectopic expression of *atjmt* in *Nicotiana attenuata*: Creating a metabolic sink has tissue-specific consequences for the jasmonate metabolic network and silences downstream gene expression. **Plant Physiol** 157: 341–354
- Varbanova M, Yamaguchi S, Yang Y, McKelvey K, Hanada A, Borochoy R, Yu F, Jikumaru Y, Ross J, Cortes D, Ma CJ, Noel JP, Mander L, Shulaev V, Kamiya Y, Rodermeil S, Weiss D, Pichersky E (2007) Methylation of gibberellins by *Arabidopsis* GAMT1 and GA MT2. **Plant Cell** 19: 32–45
- Wu JS, Wang L, Baldwin IT (2008) Methyl jasmonate-elicited herbivore resistance: Does MeJA function as a signal without being hydrolyzed to JA? **Planta** 227: 1161–1168
- Xie DX, Feys BF, James S, Nieto-Rostro M, Turner JG (1998) CO I1: An *Arabidopsis* gene required for jasmonate-regulated defense and fertility. **Science** 280: 1091–1094
- Xin ZJ, Yu ZN, Erb M, Turlings TCJ, Wang BH, Qi JF, Liu SN, YG L (2012) The broad-leaf herbicide 2,4-dichlorophenoxyacetic acid turns rice into a living trap for a major insect pest and a parasitic wasp. **New Phytol** 194: 498–510
- Yang JO, Nakayama N, Toda K, Tebayashi S, Kim CS (2013) Elicitor(s) in *Sogatella furcifera* (Horvath) causing the Japanese rice plant (*Oryza sativa* L.) to induce the ovicidal substance, Benzyl Benzoate. **Biosci Biotech Bioch** 77: 1258–1261
- Yang N, Zhu CH, Gan LJ, Ng D, Xia K (2011) Ammonium-stimulated root hair branching is enhanced by methyl jasmonate and suppressed by ethylene in *Arabidopsis thaliana*. **J Plant Biol** 54: 92–100
- Yang Y, Yuan JS, Ross J, Noel JP, Pichersky E, Chen F (2006) An *Arabidopsis thaliana* methyltransferase capable of methylating farnesoic acid. **Arch Biochem Biophys** 448: 123–132
- Ye M, Luo SM, Xie JF, Li YF, Xu T, Liu Y, Song YY, Zhu-Salzman K, Zeng RS (2012) Silencing CO1 in rice increases susceptibility to chewing insects and impairs inducible defense. **PLoS ONE** 7: e36214
- Yoshida S, Forno DA, Cock JH, Gomez KA (1976) *Laboratory Manual for Physiological Studies of Rice*. 3rd ed. International Rice Research Institute, Manila, Philippines. pp. 61–64
- Yuan JS, Kollner TG, Wiggins G, Grant J, Degenhardt J, Chen F (2008) Molecular and genomic basis of volatile-mediated indirect defense against insects in rice. **Plant J** 55: 491–503
- Zhao N, Ferrer JL, Ross J, Guan J, Yang Y, Pichersky E, Noel JP, Chen F (2008) Structural, biochemical, and phylogenetic analyses suggest that indole-3-acetic acid methyltransferase is an evolutionarily ancient member of the SABATH family. **Plant Physiol** 146: 455–467
- Zhao N, Guan J, Ferrer JL, Engle N, Chern M, Ronald P, Tschaplinski TJ, Chen F (2010) Biosynthesis and emission of insect-induced methyl salicylate and methyl benzoate from rice. **Plant Physiol Biochem** 48: 279–287
- Zhou GX, Qi JF, Ren N, Cheng JA, Erb M, Mao BZ, Lou YG (2009) Silencing OsHI-LOX makes rice more susceptible to chewing herbivores, but enhances resistance to a phloem feeder. **Plant J** 60: 638–648

## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.

**Table S1.** Primers and probes used for quantitative real-time PCR analysis of target genes.

**Figure S1.** Vectors used for enzyme activity (A), subcellular location (B) and transformation (C) used in the study.

**Figure S2.** Localization of OsJMT1 in *Nicotiana tabacum* cells. *N. tabacum* cells were transformed with OsJMT1:EGFP and EGFP. After incubation for 36 h, the transformed cells were observed

under a confocal microscope. The photographs were taken under UV light, visible light, and in combination (overlay), respectively.

**Figure S3.** Identification of *oe-JMT* lines. **(A)** Genomic DNA was digested with *EcoRI* (E) or *XbaI* (X). A fragment (about 0.7 kb) of the DNA sequence of the GUS reporter gene was used as a detection probe. Hybridization was created using the DIG High Prime DNA Labeling and Detection Starter Kit II (Roche). All three *as-pld* lines contained a single insertion of the transgene. **(B)** Expression levels of *OsJMT1* in stems of WT plants and two *oe-JMT* lines (relative to *OsACT* expression levels; mean + SE;  $n = 5$ ). Letters within the same time point indicate significant differences among different lines ( $P < 0.05$ , Duncan's multiple range test).

**Figure S4.** Phenotype of wild-type (WT) plants and *oe-JMT* lines at the seedling stage **(A)**, tillering stage **(B)**, heading stage **(C)**, and plant height at the mature stage **(D)**. Letters indicate significant differences among different lines ( $P < 0.05$ , Duncan's multiple range test).

**Figure S5.** Performance of brown planthopper (BPH) on *oe-JMT* lines and wild type (WT) plants. **(A, B)** Mean developmental duration **(A)** and death rate **(B)** (+SE) of BPH eggs. **(C–E)** Mean percentage of female adults **(C)** and developmental duration of immature stage of female adults **(D)** and male adults **(E)** (+SE). **(F)** Mean number of eggs (+SE) laid per female adult for 10 d. DD, Developmental duration.

**Figure S6.** Mean levels of MeJA **(A)**, JA **(B)**, and *OsAOS1* **(C)** and *OsHI-LOX* **(D)** transcripts (+SE;  $n = 5$ ) in wild-type (WT) plants and *oe-JMT* lines before and after SSB infestation. Letters indicate significant differences between different lines ( $P < 0.05$ , Duncan's multiple range test).

**Figure S7.** Mean levels of MeJA **(A)** and JA **(B)** (+SE;  $n = 5$ ) in wild-type (WT) plants and *oe-JMT* lines 1 h after lanolin or 50  $\mu$ g MeJA treatment. Asterisks indicate significant difference between treatments (\* $P < 0.05$ , Student's *t*-test). Letters indicate significant differences between different lines ( $P < 0.05$ , Duncan's multiple range test).